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(54) Title: USE OF SUPERCRITICAL FLUIDS TO OBTAIN POROUS SPONGES OF BIODEGRADABLE POLYMERS

(57) Abstract

A method of preparing a biodegradable porous matrix is disclosed, which comprises contacting a biodegradable polymer with a supercritical fluid in a chamber and subsequently reducing the pressure in the chamber in a sharp step. The matrix thus produced has a high porosity and may be used in a variety of pharmaceutical applications such as for surgical implantation or in controlled-release drug delivery systems.

- 1 -

USE OF SUPERCRITICAL FLUIDS TO OBTAIN POROUS

SPONGES OF BIODEGRADABLE POLYMERS

The present invention relates to the preparation of porous materials made of biodegradable polymers.

5   Polymeric matrices which are both porous and biodegradable are useful in a variety of pharmaceutical applications, such as controlled release drug delivery and surgical implantation. For example, a bone graft substitute comprising a biodegradable porous polymer is

10   described in GB-A-2215209, whilst a controlled drug delivery system comprising porous biodegradable polymeric microspheres is discussed by Sato et al in Pharm.Research, Vol.5, No.1, pp 21-30, 1988. A subcutaneously implanted porous polyvinyl alcohol sponge used to treat rats with

15   basic fibroblast growth factor (bFGF) is reported by McGee et al in J.Surgical Research 45, 145-153 (1988), which treatment is observed to accelerate wound healing in the rats.

A convenient new method of making a porous biodegradable

20   polymer has now unexpectedly been found. Accordingly, the present invention provides a method of preparing a biodegradable porous polymer matrix which comprises contacting a biodegradable polymer with a supercritical fluid in a chamber and subsequently reducing the pressure

25   in the chamber to a value below the critical pressure of the fluid in a sharp step.

- 2 -

By supercritical fluid is meant a gas or liquid above
its critical point. The critical point of a substance is
the point on a state diagram of temperature plotted against
pressure at which there ceases to be a dividing line
5   between the gaseous and liquid states. At the critical
point physical properties of the liquid and gaseous states,
in particular the densities, are identical. The
temperature and pressure values at the critical point may
be termed the critical conditions and are constant for a
10   given fluid. The critical conditions for a variety of
fluids are listed in Table 1 which follows.

TABLE 1

Critical Conditions for Various Gases and Liquids

	Critical Temperature	Critical Pressure	
	°C	kPa	(atm)
Carbon Dioxide	31.1	7376	(72.8)
Ethane	32.3	4884	(48.2)
Ethylene	9.3	5036	(49.7)
Propane	96.7	4246	(41.9)
Propylene	91.9	4620	(45.6)
Cyclohexane	280.3	4073	(40.2)
Isopropanol	235.2	4762	(47.0)
Benzene	289.0	4894	(48.3)
Toluene	318.6	4114	(40.6)
p-Xylene	343.1	3516	(34.7)
Chlorotrifluoromethane	28.9	3921	(38.7)
Trichlorofluoromethane	198.1	4408	(43.5)
Ammonia	132.5	11280	(111.3)
Water	374.2	22050	(217.6)

- 3 -

A supercritical region may be defined in the state diagram of a substance, as illustrated in Figure 1 of the accompanying drawings. A fluid is in a supercritical condition when subjected to temperature and pressure values

5   falling within this region.

Any supercritical fluid, including any of those listed above in Table 1, may be used in the process of the present invention. Carbon dioxide, at a pressure of at least 7376 kPa and a temperature of at least 31.1°C, is a preferred

10   example.

The biodegradable polymer is suitably brought into contact with the supercritical fluid in the chamber of any standard supercritical extractor or any suitable device, examples being the extractor supplied by Muller Extract

15   Company GmbH, the Sample Preparation Accessory supplied by Milton Roy, or the apparatus shown in Figure 2. In operation, the biodegradable polymer is loaded in the extraction chamber of the supercritical extractor and the supercritical fluid is then applied for a time sufficient

20   to allow supercritical fluid to penetrate the mass of the polymer by diffusion, and possibly dissolve the mass. The time needed will vary according to the fluid and the particular supercritical conditions used, and also according to the amount of material to be penetrated or

25   dissolved. The greater the mass of polymer, the longer will be the time needed for the process to be completed,

- 4 -

assuming all other parameters controlling the process to
remain constant. For a mass of polymer of from 1 to 50g,
the time is typically of the order of from 3 minutes to 2
hours. For example, when carbon dioxide is the
5   supercritical fluid it is suitably applied to this mass of
material for 45 minutes under conditions of 32°C and 7600
kPa (75 atm), or for 1 hour at 32°C and 15200 kPa (150
atm).

Once the process is complete, the pressure inside the
10 supercritical chamber is reduced in a sharp step. This
means that the pressure in the chamber is brought to a
value below the critical pressure of the fluid, typically
in a period of from 0 to 3 minutes. Preferably the period
is from 5 to 30 seconds. The pressure may be reduced in
15 this sharp step to any given value below the critical
pressure of the fluid; generally it is between the critical
pressure and ambient pressure. If this given value is
higher or lower than ambient pressure, the pressure in the
chamber is restored to ambient subsequently. In one
20 embodiment of the invention the pressure in the chamber is
reduced to ambient pressure in the sharp step. By ambient
pressure is meant a pressure of about 101.325 kPa
(1 atmosphere).

The sharp pressure reduction contributes towards
25 achieving the desired porosity in the material obtained.
The greater the difference between the operating pressure

- 5 -

of the supercritical chamber and ambient pressure, the

higher is the porosity of the resulting product. The

operating temperature of the supercritical chamber can also

be adjusted to modify the product porosity; the higher the

5   supercritical temperature employed, the higher is the

porosity achieved.

During the process of the invention the operating

temperature in the supercritical chamber is controlled.

Any suitable control means may be used, for example a

10   water-recirculating bath such as is employed in the Muller

extractor, or an air stream such as is employed in the

Sample Preparatory Accessory (SPA) apparatus supplied by

Milton Roy. The particular operating (supercritical)

temperature selected in a given situation will depend

15   partly upon the solubility of the biodegradable polymer in

the supercritical fluid since, at a given pressure, this

solubility will vary with temperature. It will also partly

depend upon the diffusion coefficient of the supercritical

fluid in the polymer since, at a given pressure, this

20   coefficient will vary with temperature. As previously

indicated, the particular operating temperature employed

affects the physical state of the polymer which is

achieved.

It is sometimes desirable to operate at the lowest

25   temperature compatible with the solubility of the polymer

in the supercritical fluid, and/or with permeation of the

- 6 -

supercritical fluid into the polymer under given
supercritical pressure conditions. This may be, for
example, when a thermally unstable active ingredient is
contained in the polymer. At other times, however, this
5   consideration does not apply and a higher operating
temperature may be selected.

Any biodegradable polymer may be used as starting
material in the process of the invention, suitable examples
including polylactides. The term polylactide designates
10 the general class of polymers which can be prepared from
one or more of the following monomers: 3-propiolactone
tetramethylglycolide, b-butyrolactone, 4-butyrolactone,
pivalolactone, and intermolecular cyclic esters of
α-hydroxy butyric acid, α-hydroxyisobutyric acid,
15 α-hydroxyvaleric acid, α-hydroxyisovaleric acid,
α-hydroxycaproic acid, α-hydroxy-α-ethylbutyric acid,
α-hydroxyisopcaproic acid, α-hydroxy-3-methylvaleric acid,
α-hydroxyheptanoic acid, α-hydroxyoctanoic acid, α-
hydroxydecanoic acid, α-hydroxymyristic acid,
20 α-hydroxystearic acid, and α-hydroxylignoceric acid. It is
most preferred to use lactic acid as sole monomer or lactic
acid as the principal monomer with glycolic acid as the
comonomer. The latter are termed poly(lactide-co-
glycolide) copolymers.

25   Particularly suitable are polymers prepared from lactic
acid alone, glycolic acid alone, or lactic acid and

- 7 -

glycolic acid wherein the glycolic acid is present as a
comonomer in a molar ratio of 100:0 to 40:60. It is most
preferred to use a poly(lactide-co-glycolide) copolymer
having a molar ratio between about 80:20 and 50:50.

5   Poly(lactide) homopolymers, poly(glycolide) homopolymers
and poly(lactide-co-glycolide) copolymers may range in size
from 1,000 to 200,000 in molecular weight stated as an
average ($\overline{MW}$). The molecular weight of a particular
10 copolymer is independent of its monomeric makeup. For
example, a 50:50 copolymer can have a molecular weight
which falls anywhere within this range. Therefore polymers
can be varied both as to their monomer composition as well
as their molecular weight.

15   Examples of other suitable biodegradable polymers
include: polyanhydrides, for example as described in
US-A-4,757,128, Biomaterials 1983, Vol. 4, pages 131-133,
J. Biomedical Materials Research, Vol. 19, pages 941-955
(1985), J. Biomedical Materials Research, Vol. 20,
20 pages 51-64 (1986) or Biomaterials 1986, Vol. 7,
pages 364-371; and polyacetals, polyketals and
poly(orthoesters), for example as described in
Polym.Sci.Technol. 1986, Vol. 34, Polymer Med. 2,
pages 357-365.

25   The biodegradable polymeric starting material may be in
the form of microspheres, pellets, a matrix of any
geometrical shape, or in any other form. When microspheres

- 8 -

or any other of the aforementioned starting materials are
used these may be, if desired, pre-loaded with an active
ingredient so that the process of the invention yields, in
one step, a porous spongy material carrying an active
5   ingredient. Microspheres of a poly(lactide-co-glycolide)
copolymer or of a polylactide homopolymer are particularly
suitable.

When the biodegradable polymer is in the form of
microspheres, these are prepared in a conventional manner.
10   A standard method involves dispersing the biodegradable
polymer in an organic solvent, a suitable example being
dichloromethane, and adding a silicone oil dropwise to the
dispersion with stirring. The microspheres thus obtained
are then washed and hardened by treatment with a non-polar
15   hydrocarbon solvent such as pentane or heptane.

The active ingredient contained in the microspheres may
be any drug used in surgery, therapy or prophylaxis.
Examples include: peptides and proteins, in particular
immunomodulators such as Thymic Humoral Factor; growth
20   factors such as basic Fibroblastic Growth Factor, acid
Fibroblastic Growth Factor, Epidermal Growth Factor, Human
Growth Factor, Insulin Like Growth Factor, Platelet Derived
Growth Factor, Nerve Growth Factor and Transforming Growth
Factor; antitumorals such as BCNU or 1,3-bis(2-chloro-
25   ethyl)-1-nitrosourea, daunorubicin, doxorubicin,
epirubicin, idarubicin, 4-demethoxydaunorubicin

- 9 -

3'-desamine-3'-(3-cyano-4-morpholinyl) - doxorubicin,

4-demethoxydaunorubicin-3'-desamine-3'-(2-methoxy-4-

morpholinyl)-doxorubicin, etoposide and teniposide;

hormones such as LHRH and LHRH analogues; and steroideals

5   for birth control and/or antitumoral action such as

medroxyprogesterone acetate or megestrol acetate.

If forms of biodegradable polymer other than

microspheres are used, such as pellets or matrices of any

geometric shape, these may also, if desired, be pre-loaded

10 with an active ingredient such as any of those listed

above.

The porous biodegradable polymeric material obtained in

accordance with the invention may be used as a surgical

implant, for example to aid the reconstitution of tissues

15 after surgical removal of a tumour or after trauma

injuries. Such an implant has immediate utility in filling

anti-aesthetic voids caused by the surgery or injury,

giving support for newly-growing tissue which is able to

fill the pores of the material. The biodegradable material

20 itself, as it disintegrates in vivo, will then gradually be

replaced by the new tissue. The time required for the

degradation of the biodegradable material is related to the

composition of the polymer(s), the molecular weight of the

polymer, the relative proportions of comonomers (in the

25 case of a copolymer(s)), and the physical state. The

process of reconstitution of the damaged tissue is enhanced

- 10 -

if the porous polymer is loaded with a suitable growth
factor such as any of those listed earlier, chosen
according to the particular application.

The porous biodegradable material is also particularly
5   useful in the treatment of tumours which are not removable.
In this case the material is loaded with an anti-tumour
agent, such as any of those listed earlier, and implanted
at the site of the tumour. An example is the treatment of
the glioblastoma multiform in the brain.

10 The porosity (P) of the material produced in accordance
with the invention may be calculated, in percentage terms,
using the following equation:

$$P = [1-(D1/D2)] \times 100$$

in which D1 is the apparent density of the material and D2
15 is the true density, measured by the standard method of
helium picnometry. A porosity of up to as high as 74% is
achieved in accordance with the invention.

Besides giving rise to a highly porous product the
process of the invention has the important advantages that,
20 since it allows a non-oxidising agent to be used at a
relatively low temperature (e.g. CO_2 above 31.1°C), it is
relatively non-hazardous and non-toxic and does not cause
degradation of any active ingredient contained in the
biodegradable polymer starting material. In addition, it
25 is easy to carry out on a large scale and is therefore of
economic importance.

- 11 -

The following Examples further illustrate the invention.

Reference Example 1: Preparation of biodegradable microspheres

2g of poly (D,L) lactide, of molecular weight 100,000
5   and intrinsic viscosity of 1 (Boehringer Ingelheim) were
dissolved in 80 ml of CH_2Cl_2. 0.8 ml of phosphate buffer
(pH 7.4, I=0.1) were dispersed finely in the CH_2Cl_2 phase
with an Ultra-Turrax stirring turbine equipment, operating
at 4000 rpm with a dispersing tool (type G45F). 60ml of
10   silicone oil Dow Corning Fluid 200 were added dropwise
under continued stirring.

The microspheres obtained were washed and hardened with
21 of n-heptane, then desiccated under vacuum at 35°C for
24 hours, and sieved. The microspheres obtained had a mean
15   diameter of about 80 μm.

The n-heptane residual content was about 17%.

Reference Example 2

The process of Reference Example 1 was carried out using
poly ((D,L) lactide-co-glycolide) copolymer, having a
20   lactide: glycolide ratio of 75:25, a molecular weight of
18000 and intrinsic viscosity of 0.8 (Boehringer Ingelheim)
instead of the poly (D,L) lactide.

The n-heptane residual content was about 10%.

- 12 -

Reference Example 3

The process of Example 2 was repeated using n-pentane in place of n-heptane.

The n-pentane residual content was about 5%

5   Reference Example 4: Preparation of biodegradable micro-spheres loaded with an active ingredient

2g of poly (D,L) lactide of molecular weight 100,000 and intrinsic viscosity of 1 (Boehringer Ingelheim) were dissolved in 80ml of CH_2Cl_2. 200mcg of basic fibroblast

10 growth factor was dissolved in 0.8ml of phosphate buffer (pH 7.4, I=0.1) and the aqueous phase dispersed finely in the CH_2Cl_2 with an Ultra-Turrax stirring turbine apparatus, operating at 4000 rpm with a dispersing tool (type G45F). 60ml of silicone oil Dow Corning Fluid 200 were added

15 dropwise under continous stirring. The microspheres obtained were washed and hardened with 2l of n-heptane, then desiccated under vacuum at 35°C for 24 hours, and sieved.

The microspheres obtained had a mean diameter of about

20 80 μm. The n-heptane residual content was about 17%.

EXAMPLE 1: Preparation of porous biodegradable material

1.5 g of the microspheres obtained by the process of Reference Example 1 were loaded in the extraction chamber of a supercritical extractor (Muller Extract Company GmbH).

- 13 -

CO_2 was applied at 15200 kPa (150 bar) and 35°C for 1 hour.

The CO_2 was taken from a bomb (cylinder) and loaded into the extractor. The pressure of 15200 kPa (150 bar) was applied by means of a mechanical pump. The temperature was
5 kept at 35°C during the process by means of a recirculating water bath. After 1 hour the pressure was reduced to ambient pressure in 10 seconds. The extraction chamber was opened and a spongy material was obtained.

The porosity of the material was determined by the
10 equation:

$$P = [1-(D1/D2)] \times 100$$

where D1 is the apparent density and D2 is the true density measured by helium picnometry and found to be about 76%.

The residual content of n-heptane was 100 ppm.

15 EXAMPLE 2

The process of Example 1 was repeated but using the following CO_2 supercritical conditions: 7600 kPa (75 bar), 32°C, 45 min.

The material obtained was found to have a porosity of
20 about 64%.

EXAMPLE 3

The process of each of Examples 1 and 2 was repeated, but using the biodegradable microspheres loaded with active ingredient prepared in Reference Example 4. A spongy bio-

- 14 -

degradable materials loaded with the active ingredient was

obtained, in each case, having a porosity analogous to that

achieved with the unloaded material obtained in Examples 1

and 2.

5   Reference Example 5

The process of Example 1 was repeated, but using the

following CO_2 subcritical conditions: 5066 kPa (50 bar),

32°C, 15 min. The material obtained was characterized with

a porosity of about 54%.

- 15 -

CLAIMS

1.   A method of preparing a biodegradable porous matrix
which comprises contacting a biodegradable polymer with a
supercritical fluid in a chamber and subsequently reducing
5   the pressure in the chamber to a value below the critical
pressure of the fluid in a sharp step.

2.   A method according to claim 1 wherein the
biodegradable polymer is a polylactide homopolymer or a
poly(lactide-co-glycolide) copolymer.

10   3.   A method according to claim 1 or 2 wherein the
biodegradable polymer is in the form of microspheres.

4.   A method according to any one of the preceding
claims wherein the biodegradable polymer is loaded with an
active substance.

15   5.   A method according to any one of the preceding
claims wherein the supercritical fluid is carbon dioxide at
a temperature of at least 31.1°C and a pressure of at least
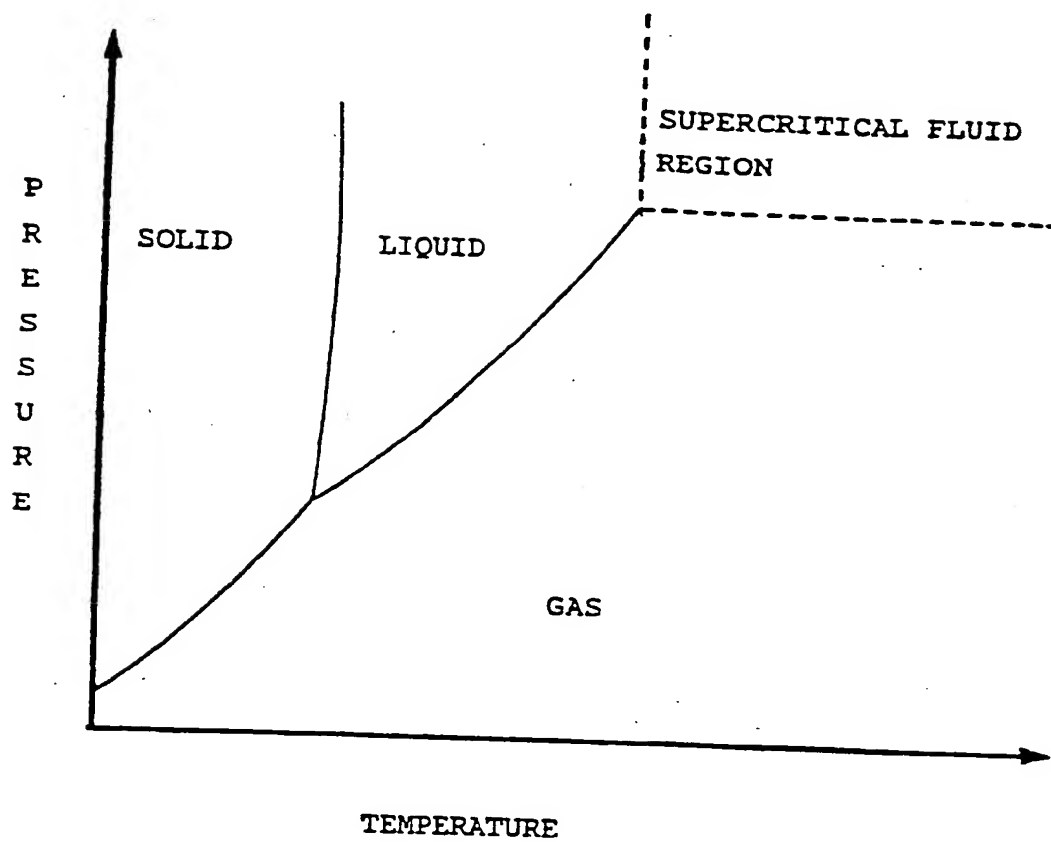72.8 atmospheres.

6.   A surgical implant comprising a biodegradable
20   porous matrix prepared by the method as claimed in claim 1.

7.   A controlled release drug delivery system
comprising a biodegradable porous matrix prepared by the
method as claimed in claim 1 and loaded with an active
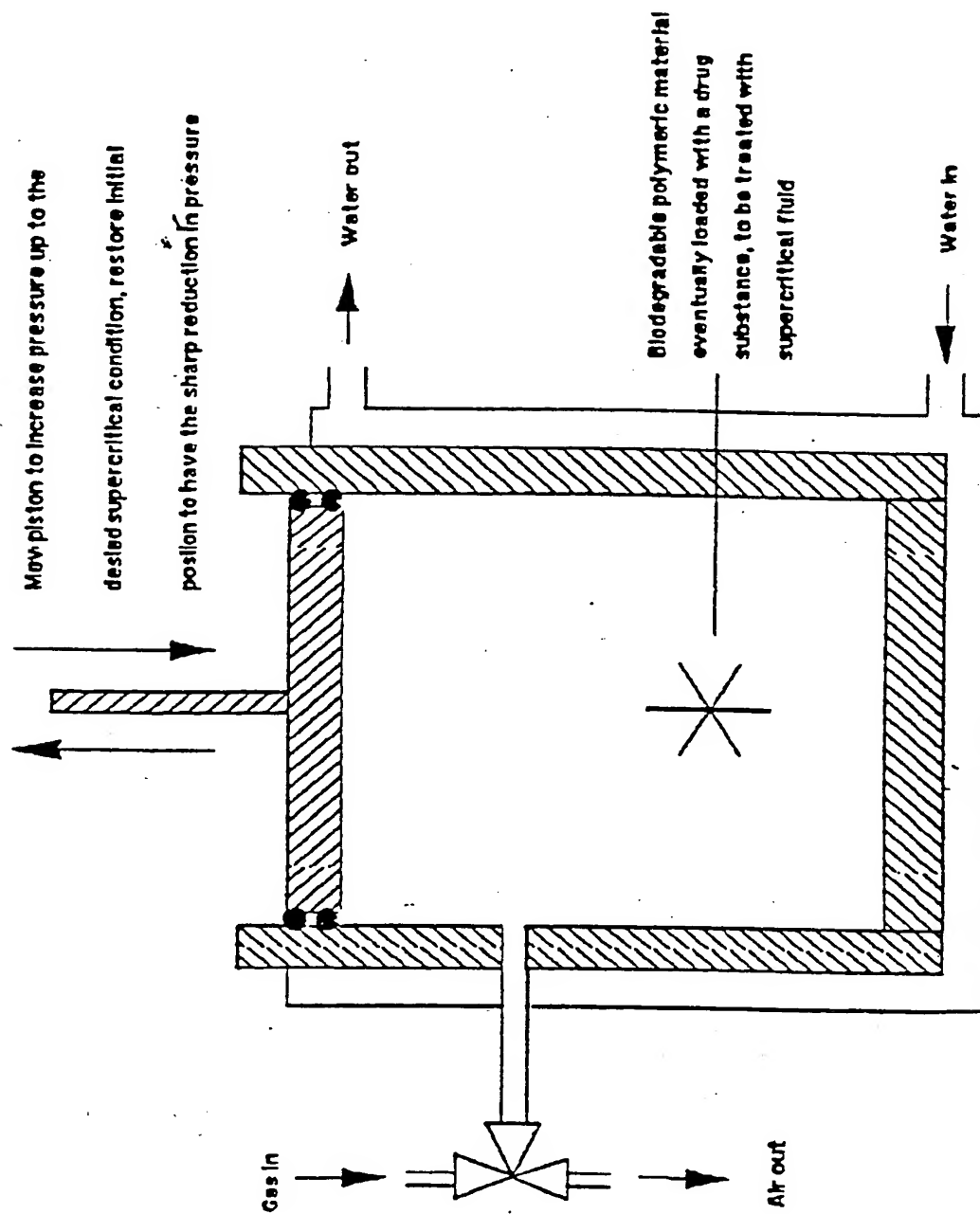ingredient.

8.   A method of preparing a biodegradable porous matrix
substantially as hereinbefore described in any one of
Examples 1 to 4.

1/2

FIGURE 1

212

Move piston to increase pressure up to the

desired supercritical condition, restore initial

position to have the sharp reduction in pressure

Water out

Biodegradable polymeric material
eventually loaded with a drug
substance, to be treated with
supercritical fluid

Water in

Gas in

Air out

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No **PCT/EP 90/01895**

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5   C08J9/18 ;   A41K9/16 ;   //C08L67:04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C08J ; A41K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[o]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	EP,A,113903 (JAPAN STYRENE PAPER CORPORATION) see page 2, line 29 - page 2, line 37; claims 1-5, 9, 10 ---	1-8
Y	US,A,4719246 (DU PONT DE NEMOURS) see column 4, line 65 - column 5, line 3; claims 1-16 ---	1-8
A	EP,A,251631 (ANDERSON) see claims 1-10 ---	1-8
A	EP,A,204476 (INTERNATIONAL MINERALS AND CHEMICALS CORP.) see claims 1-15 ---	1-8
A	US,A,3681270 (DYNAMIT NOBEL A.G.) see claims 1, 2, 6 ---	1-8

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31 JANUARY 1991	1 8 FEB 1991
International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	OUDOT R.

Form PCT/ISA/210 (second sheet) (January 1985)

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		JP-A-	63241024	06-10-88
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US-A-3681270	01-08-72	None		

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